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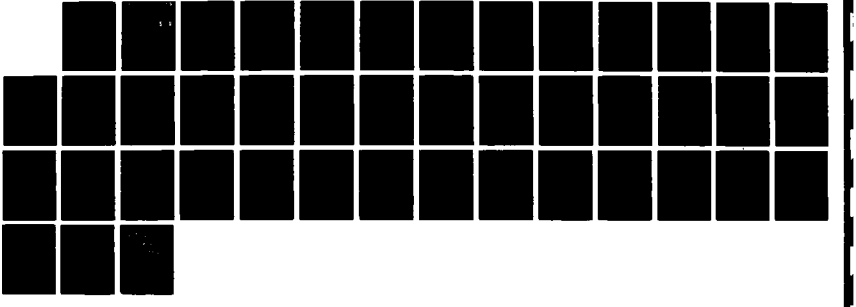

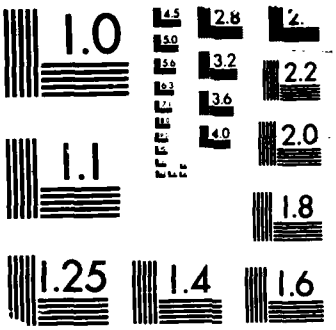

A PROGRAM FOR THE STUDY OF SKELETAL MUSCLE CATABOLISM FOLLOWING PHYSICAL TRAUMA

ANNUAL REPORT

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The purpose of this work was to attenuate skeletal muscle proteolysis in the post-traumatic period. In the initial study, amino acid solutions were administered with or without glutamine supplimentation. Amino Acid administration at the dose of 0.624 grams N/kg/hour was associated with near nitrogen balance, maintenance of skeletal muscle intracellular stores, and attenuation of hindquarter nitrogen loss.

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I. SUMMARY

The purpose of this study was to attenuate skeletal muscle proteolysis in the postraumatic period. In the initial study, amino acid solutions were administered with or without glutamine supplementation. Amino acid administration at the dose of 0.624 grams N/kg . hour was associated with near nitrogen balance, maintenance of skeletal muscle intracellular stores, and attenuation of hindquarter nitrogen loss. Glutamine enriched solutions were as effective as standard balanced formulas in sparing body protein.

In a second study, amino acid formulas were constructed to provide a range of concentrations of branched chain amino acids (from 12%-44%). Isonitrogenous infusions were administered to animals following operations and the effects of the various formulas determined. No benefit was derived from branched chain enriched solutions over a standard formula.

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In additional studies, adrenergic blockade was achieved by administering phentolamine and propranolol or utilizing high epidural anesthesia. While blockade did not reduce nitrogen excretion in the posttraumatic period, nitrogen efflux from the hindquarter was markedly attenuated. This is the first demonstration of a relationship between the adrenergic nervous system and accelerated proteolysis. The significance of these findings is discussed.

FOREWORD

In conducting the research described in this report, the investigator adhered the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

III. The Effect of Supplemental Amino Acids in Altering Skeletal Muscle Proteolysis

A. Glutamine Administration

Accelerated nitrogen excretion following moderate or severe injury was first described by Cuthbertson over 50 years ago (1) and has since been recognized as a characteristic feature of the metabolic response to trauma. The site of the protein loss was thought to be skeletal muscle, an hypothesis that was initially confirmed by Levinson, who demonstrated the translocation of skeletal muscle nitrogen to visceral protein in experiments using 15N-labeled amino acids in injured rats (2). Subsequent investigations in humans documented an increased skeletal muscle release of amino acids from the uninjured extremities of thermally injured patients (3), a finding that corresponded with a marked uptake of amino acids across the splanchnic bed (4).

With the development of microanalytic techniques and methodology for sampling human skeletal muscle and other tissues, it has been possible to define the alterations in plasma and free amino acid pools in various tissues following injury (5). Whole blood and skeletal muscle intracellular glutamine concentrations have been found to decline markedly in response to a variety of stresses (6), including sepsis (7) and elective operations (8). These alterations occur even when solutions containing glucose and amino acids are infused (9). The restoration of muscle intracellular glutamine levels is slow, with a return to normal concentrations, possibly signaling an end to post-injury convalescence. The decline in muscle glutamine is especially significant, since 80% of the free amino acids of the body reside within the intracellular compartment of skeletal muscle, and glutamine alone accounts for approximately two-thirds of this free amino acid pool (excluding taurine) (10). Whole blood glutamine concentrations also are higher than levels of any other amino acid. Although a storage form of body nitrogen is not generally recognized, muscle and plasma glutamine represents a large free amino acid pool that appears to be an important available form of amino acid nitrogen (11).

Intracellular glutamine concentrations in skeletal muscle decrease rapidly in the dog following a standard surgical procedure, similar to the response observed in humans (12). There is a 40% fall in intracellular glutamine concentrations within 24 hours of standard operation; this depression is maintained for 72 hours and returns gradually to normal by the eighth postoperative day. Studies in pair-fed animals have demonstrated that these postoperative alterations are not the result of short term nutritional depletion. The present study was designed: (a) to determine if the postoperative decline in muscle glutamine concentrations in dogs could be prevented by the infusion of glutamine and/or standard intravenous amino acid solutions, and (b) to characterize the influence of amino acid infusion on nitrogen balance and on the efflux of amino acids from the hindquarter skeletal muscle.

1. Materials and Methods

Animal Care and Operative Procedures

Twenty-two mongrel dogs, weighing between 20 and 40 kg, were obtained from a farm where they had been regularly exercised and screened for parasites. All female animals were nonpregnant. While in our kennel, they were maintained in accordance with the guidelines of the Committee on Animals at Harvard Medical School and the Committee on Care and Use of Laboratory Animals of the Institute for Laboratory Animal Resources, the National Research Council (DHEW Publication #NIH 78-23, reviewed 1978). The animals were kept in individual kennels at a constant temperature of 20°C, with 24-hour light exposure. They were exercised for two hours every morning, provided with water ad lib, and given a single daily feeding between 1:00 and 3:00 p.m. of Agway Respond 2000 Dry Dog Chow® (contains at least 25% protein, 10% fat, and the remaining calories as carbohydrate). Five to seven days were allowed for the dogs to acclimate to the kennel conditions, during which time they were trained to rest quietly in a Pavlov stand. On the day before obtaining basal samples, all food was removed from the kennel at 5:00 p.m. After an overnight fast, the dog was walked for at least 20 minutes, placed in a Pavlov stand, and a foreleg vein was cannulated. After the dog had rested in the stand for at least 20 minutes, a venous blood sample was obtained for amino acid determination. Following rapid induction of anesthesia with intravenous sodium thiopental (Abbott Laboratories, 5 mg/kg body weight), a biopsy of the vastus lateralis

muscle was obtained by the method of Bergstrom (5). The animal was then taken out of the stand and a 5-ml sample of arterial blood was obtained from the femoral artery via percutaneous puncture.

The animal was allowed to recover from the biopsy for a minimum of two days prior to the standard operative procedure. On the day prior to surgery all food was again removed from the kennel at 5:00 p.m. At 7:00 a.m. the dog was walked for 20 minutes and then taken to the operating suite where it was anesthetized with intravenous sodium pentobarbital (Abbott Laboratories, 30 mg/kg body weight). An endotracheal tube was placed, and the animal was allowed to spontaneously breathe a mixture of oxygen and room air. The dog was placed on an operating table in a supine position, and a cannula placed by percutaneous puncture into the external jugular vein and directed into the superior vena cava. After noting the starting time, the infusion solution was administered via this cannula by constant infusion (IMED pump®, San Diego, CA) at 4 ml/hour · kg. Penicillin G (E.R. Squibb, Princeton, NJ; 600 mg) and Keflin® (Eli Lilly, Indianapolis, IN; 1 gram) were given intravenously. The urinary bladder was catheterized, the initial urine sample discarded, and the catheter was connected to a closed urine bag for 24-hour collection. The abdomen and flanks of the dog were shaved, and the skin washed with soap and water, and prepared with a povidone iodine prep solution (Clinipad Corporation, Guilford, CT). The dog was draped with sterile sheets and the abdominal cavity entered via a vertical infra-umbilical incision in females and a right paramedial incision in males. The bowel was retracted into the upper abdomen, and the exposed retroperitoneum incised. The right deep circumflex iliac artery and vein and the medial sacral artery were isolated by sharp and blunt dissection. A specially prepared catheter consisting of a 6-cm segment of polyethylene tubing (2.08 mm OD) coated with silastic and linked to a 2.8 mm OD polyethylene catheter was inserted 6 cm cranially into the aorta via the right deep circumflex iliac artery. A similar catheter was inserted into the middle sacral artery, its tip being positioned approximately 1 cm proximal to the bifurcation of the aorta, but distal to the inferior mesenteric artery. A third catheter was inserted into the inferior vena cava via the right deep circumflex iliac vein and positioned distal to the renal vein. All catheters were secured and exteriorized through stab wounds in the flank. The abdomen was then closed, and the animal was turned onto its left side. The exterior catheters were cut to appropriate lengths, plugged with blunt needles connected to intermittent injection ports (Jelco®,

Critikon, Inc., Tampa, FL), flushed with saline, filled with heparin (1,000 units/ml), and buried subcutaneously. The injection ports were positioned high on the flank of the animal under the skin and in the approximate vicinity of the vertebral column. This allowed access to the aorta and vena cava by percutaneous puncture of the injection ports of the catheters. Two further doses of Keflin ® (1 gram) were given 8 and 24 hours postoperatively via the venous catheter.

Following the operative procedure the animal was placed on its side, and body temperature was maintained with heat lamps and blankets during recovery from anesthesia. Approximately five hours after the start of the infusion the animal was placed in a Pavlov stand, and a solution of para-aminohippuric acid (PAH, 0.5% w/v in saline) was infused at a rate of 0.76 ml/minute with a Harvard pump into the distal aorta through the medial sacral artery catheter. After 40 minutes of dye infusion, simultaneous arterial and venous samples were obtained for measurement of amino acid and PAH concentrations. Three sample sets were drawn at 10-minute intervals over a period of 20 minutes. The catheters were then flushed, filled with heparin, and the animal was kept in a Pavlov sling. Twenty-three hours following the initiation of the experiment, the hindquarter flux studies were repeated. After 24 hours, the urine collection was terminated. The animal received intravenous sodium thiopental, as previously described, and biopsy of the vastus lateralis muscle in the leg not previously biopsied was performed. The intravenous infusion was terminated, and the animal placed in a metabolic cage for the ensuing 24 hours where it was offered water ad lib and no food.

Infusion Solutions

All animals received an infusion at a rate of 4 ml/hour · kg. Five control animals received 0.9% saline. Other animals were given commercially available amino acid solution (FreAmine III®, American McGaw) at two different concentrations designed to deliver approximately 0.312 (N=2) or 0.624 (N=6) grams of nitrogen/24 hr · kg body weight. The higher dose was designed to provide the equivalent of 4 grams of protein/24 hr · kg body weight. Three animals received a solution containing glutamine at 0.312 grams nitrogen/24 hr · kg. A final group (N=6) received an equal mixture of glutamine and FreAmine , providing nitrogen at 0.624 grams/24 hr · kg. The glutamine solutions were made by dissolving L-glutamine (Sigma, St. Louis, MO) in distilled water to form a 0.157 M solution which was then adjusted to pH 6.8 with sodium hydroxide. This solution was sterilized by filtration through a 0.22 μM membrane and stored at 4°C for less

than 24 hours. On the morning of utilization the solutions were formulated at required concentrations in 2-liter bags (American McGaw) and maintained at 4°C until use. A 10-ml sample was taken from each bag at the end of the infusion and stored at -20°C for analysis of nitrogen content. An additional 10-ml sample was adjusted to pH 4.75 as described below and stored frozen for analysis of glutamine content.

Preparation and Analysis of Blood and Tissue Samples

Whole blood and plasma samples were deproteinized by combining with equal volume of ice cold 10% (w/v) perchloric acid and then centrifuging at 3000 rpm at 4°C for 20 minutes. A 2-ml aliquot of the supernatant was buffered with 0.3 ml of 2N sodium acetate (pH 4.75), adjusted to pH 4.75-4.90 with 5N potassium hydroxide, and brought to a final volume of 4 ml with distilled water. The samples were stored at -20°C for later batch analysis of glutamine and glutamate concentrations, with an enzymatic microfluorometric assay modified from the method of Lund (13).

During the muscle biopsy procedure, a stop watch was started immediately when the tissue was removed. The muscle was dissected free of fat and connective tissue and divided into two unequal portions. Both samples were weighed at least four times over the ensuing two minutes, and the weight and time following biopsy were recorded. Actual muscle wet weight at time zero was calculated from the best fit linear regression of weight plotted against time. The smaller sample (approximately 15 mg) was dried to a constant weight in a 90° oven, and the weight of dry, fat-free solids was obtained after extraction in petroleum ether. This sample was then digested in 250 µl of 1N nitric acid, and the chloride content was measured by titration with silver nitrate using a semi-automated titrator (Radiometer, Copenhagen). Plasma chloride was also determined and intra- and extracellular water calculated by the method of Bergstrom (5). The second muscle sample (approximately 100 mg) was homogenized in 0.5 ml of ice cold perchloric acid (10% w/v) with a Polytron Homogenizer (Brinkman, Westbury, NY). The homogenate was centrifuged and the supernatant prepared for enzymatic glutamine and glutamate analysis.

At the start of this study plasma and intracellular glutamine and glutamate concentrations were determined by an enzymatic method previously described (11). Concentrations of other amino acids were determined by automated high performance liquid chromatography (HPLC) after pre-column derivatization with o-phethaldehyde. All amino acids commonly found in proteins were quantitated except glutamine, glutamate, proline, cystine, and lysine. As the study progressed, techniques were developed for glutamine-glutaminate measurement using HPLC. Samples measured by the two techniques (enzymatic and HPLC) yielded comparable glutamine-glutamate concentrations; hence, only HPLC analysis was utilized in the latter portion of the study. The concentration of PAH in the arterial and venous samples was determined spectrophotometrically following deproteinization with 5% trichloroacetic acid (11).

Nitrogen Determinations

Urine excreted during the 24 hours of infusion was collected in the closed urinary collecting system and stored in acidified, refrigerated containers. Aliquots were stored frozen at -20°C for batch analysis. The nitrogen content of the infusion solution and urine was determined in the same batch by the macro-Kjeldahl method (14).

Statistical Analysis and Calculations

Statistical calculations were performed on an IBM 4341 Computer utilizing a standard statistical package (Minitab, The Pennsylvania State University, State College, PA, 1983). The results are expressed as mean±SEM. Paired and unpaired Student t-tests were used as appropriate. Analysis of variance was used for multiple group comparisons. Regression analysis was performed using methods of least squares. Because of the small sample size in the groups receiving 0.312 grams of nitrogen/24 hr · kg, most statistical comparisons were only performed between the other groups.

Hindquarter bloodflow was calculated as previously described (11), and the rate was expressed per kg body weight to account for variation in size of the animals. Amino acid flux rates were calculated as the product of bloodflow and arterial-venous concentration differences. Three sets of samples were drawn, flux was calculated for each set, and the mean of the three values determined (11). Total amino acid nitrogen in whole blood, plasma, and intracellular water was calculated by taking into account the nitrogen content of each amino acid and summing the individual differences.

2. Results

Plasma and Intracellular Amino Acid Concentrations

Plasma amino acid concentrations were measured preoperatively and 24 hours following the standard operation. In the saline-treated animals, the total nitrogen content of the plasma was unchanged by the operative procedure (Table I). The glutamine concentration remained constant, but the branched chain amino acids rose, the sum of their concentrations increasing from 326±21 to 501±9 μmol/l ($p < 0.01$). In the animals receiving 0.624 grams N/24 hr · kg, there was an upward trend in the plasma nitrogen concentration that was statistically significant only in the group receiving the mixture of amino acids plus glutamine. The plasma glutamine concentration also rose in this group. Branched chain amino acids were elevated in all animals receiving amino acid infusions.

Skeletal muscle nitrogen concentrations declined during saline infusion (Table II). This decrease in total amino acid nitrogen was reflected primarily by a fall in glutamine from 21.48±3.21 μmol/l intracellular water to 15.86±3.80 ($p < .05$). Although the sum of the concentrations of non-essential amino acids diminished, the sum of total essential amino acids in the intracellular pool remained unchanged. No change in intracellular nitrogen or glutamine occurred in animals receiving the larger dose of amino acid nitrogen (Table II). There was an upward trend in the intracellular concentration of branched chain amino acids with infusion of the higher amino acid loads, although statistical significance was achieved only in the animals receiving the mixture of amino acids and glutamine. There was not a significant change in the total concentrations of essential and non-essential amino acids in these two groups following operation. In contrast to the animals receiving the higher dose of nitrogen, the five animals infused with 0.312 gm N/24 hr · kg did not consistently maintain the skeletal muscle intracellular nitrogen pool, regardless of the solution infused. Intracellular glutamine fell in three of the animals, remained unchanged in one, and increased in one (data not shown).

Thus, providing amino acids at 0.624 gm N/24 hr · kg as an amino acid mixture with or without glutamine, maintained the skeletal muscle intracellular amino acid pool. A decrease in the intracellular pool, which was characterized by a fall in intracellular glutamine, occurred consistently in the animals receiving saline and was variable in the animals receiving the lower dose of amino acids.

Hindquarter Nitrogen Flux

Net hindquarter amino acid flux, calculated as the sum of the nitrogen flux of the individual amino acids, averaged -19.05±4.06 μmol N/minute · kg when measured at 6 hours post-operation in the animals receiving saline. This was significantly greater than the efflux rates of -7.70±5.90 and -6.50±1.81 μmol N/minute · kg observed in the two groups of animals receiving the higher doses of amino acids (Table III). However, glutamine efflux from the hindquarter was unchanged among these three groups. In contrast, branched chain amino acids were released in the saline dogs, but taken up in both groups of animals receiving the higher doses of amino acids. Hindquarter exchange of branched chain amino acids appeared to be related to the rate of branched chain amino acid administration; the hindquarter demonstrated branched chain amino acid release in the saline-treated group, balance with the solution containing amino acids plus glutamine, and greater uptake in the group receiving the highest branched chain amino acid dose. In the five animals receiving 0.312 gm N/24 hr · kg, there was not a significant alteration in hindquarter nitrogen efflux compared to the saline-treated dogs. However, there was considerable variation in these flux data, and the number of animals studied was small. Hindquarter amino acid flux studies 24 hours following operation demonstrated no differences between groups (Table III).

Nitrogen Excretion

Nitrogen excretion in the five animals infused with saline was 0.492±0.022 gm N/24 hr · kg. In the 6 animals receiving the highest dose of the commercial amino acid mixture, measured nitrogen intake was 0.632±0.001 gm N/24 hr · kg, and nitrogen excretion averaged 0.684±0.031 (Table IV). In the 6 animals receiving the solution made up of one-half commercial amino acid solution and one-half glutamine, nitrogen intake was comparable but excretion was greater, averaging 0.775±0.019 gm N/24 hr · kg ($p < 0.05$). Nitrogen balance in these two groups was significantly less negative than in the animals receiving saline, averaging -0.052±0.031 and -0.140±0.022 gm N/24 hr · kg, respectively. In the five animals that received approximately 0.312 gm N/24 hr · kg, the average nitrogen excretion was intermediate between that observed in the saline controls and in the animals receiving the larger quantity of infused nitrogen. Taken together, these studies demonstrated that nitrogen balance approached equilibrium as the quantity of administered nitrogen increased (Figure). When glutamine was combined with a commercial glutamine-free amino acid solution, the effects on nitrogen balance were additive. When summed together, the nitrogen retained in response to the infusion of commercial amino acids or glutamine alone accounted for the nitrogen retained when the solutions were combined.

3. Discussion

Operative stress in dogs stimulates net skeletal muscle protein breakdown, as evidenced by negative nitrogen balance and increased amino acid efflux from the hindquarter in association with a fall in the intracellular skeletal muscle free amino acid pool. Previous studies have demonstrated that protein wasting is not related to fasting or anesthesia, but is clearly a response to the operative stress (12). The release of amino acids from the hindquarter 6 hours postoperation in the saline-treated group was approximately 6 to 8 times that observed in chronically-catheterized, postabsorptive dogs studied under basal conditions (11). Moreover, the rate of hindquarter nitrogen release cannot be accounted for by depletion of the intracellular free amino acid pool and therefore must reflect net skeletal muscle proteolysis.

Provision of amino acids in the perioperative period offset the nitrogen loss, maintained or increased plasma amino acid concentrations, and diminished the fall in the skeletal muscle intracellular free amino acid pool. These effects appear to be related to the quantity of amino acid nitrogen infused. Whole body and hindquarter nitrogen losses were greatly decreased at the highest amino acid doses, which also maintained intracellular pools of glutamine and other amino acids. These results differ from the findings reported by Askanazi and associates (9), who described a decline in the intracellular concentrations of glutamine and other amino acids in patients after hip replacement that could not be reversed by the infusion of dextrose and amino acids. Our study would suggest that this may in part be related to the quantity of amino acids infused and/or the lack of glutamine in the infusate. Infusion of lower concentrations of amino acids (0.312 gm N/24 hr · kg), either as glutamine alone or as FreAmine®, failed to maintain the intracellular amino acid pool in three of the five animals studied. In contrast, the higher rate of amino acid infusion stabilized or increased the intracellular pool. Thus, it appears that an adequate quantity of administered nitrogen can maintain the skeletal muscle intracellular amino acid pool postoperatively.

The change in the intracellular free amino acid pool in saline-infused animals, largely attributable to a rapid fall in glutamine, was prevented when adequate nitrogen was provided. This occurred even when glutamine was not present in the commercially available solution. The mechanism by which intracellular glutamine was maintained

under these circumstances is unclear, although it seems probable that glutamate substrate for glutamine synthesis was derived from the branched chain amino acids via transamination. For unexplained reasons, net glutamine efflux was similar in all groups. Hindquarter release was not accelerated by branched chain amino acids or attenuated by the provision of glutamine in the amino acid solution. The results in this postoperative model differ from reported effects of branched chain amino acid infusion in normal humans, in whom branched chain amino acid forearm uptake was associated with accelerated glutamine release (15).

Although there were marked differences in composition of the two amino acid solutions administered at the rate of 0.624 gm N/ 24 hr · kg, hindquarter nitrogen efflux was comparable in both groups of animals. This occurred even though the quantity of essential amino acids and branched chain amino acids in the balanced solution was twice that in the glutamine-containing solution. Thus, in this experimental model of operative stress, glutamine supplementation of a balanced amino acid formula was at least as effective as standard balanced formula in diminishing hindquarter nitrogen loss.

In the dogs that received saline, branched chain amino acids were released from skeletal muscle. Quantitative transfer rates calculated from these data suggest that a marked uptake of branched chain amino acids must have occurred in visceral organs, most probably the liver, during the early postoperative period. The provision of branched chain amino acids appeared to offset this translocation by perhaps both meeting visceral requirements and reversing skeletal muscle efflux. A quantitative relationship also existed between hindquarter nitrogen balance and preservation of the intracellular nitrogen pool. When intracellular pools were maintained, the hindquarter was near nitrogen equilibrium; when saline was administered, amino acid concentrations in the intracellular pool were markedly depleted and there was a marked loss of hindquarter nitrogen. Although the relationship between skeletal muscle proteolysis and nitrogen concentration in the free amino acid pool is unknown, these data suggest that skeletal muscle nitrogen balance is related to the intracellular amino acid concentration. Further studies are necessary to determine if this relationship is causal or circumstantial.

B. Branched Chain Amino Acid Supplementation

As previously noted, accidental injury, major operative procedures, and sepsis are characterized by negative nitrogen balance, skeletal muscle amino acid efflux, and a fall in intracellular free amino acid concentrations. Branched chain amino acids (BCAA = leucine, isoleucine, and valine) are the only essential amino acids that are primarily oxidized in skeletal muscle (16). The amino group is transaminated with pyruvate or α-ketoglutarate to synthesize alanine or glutamate (17). This reaction yields branched chain ketoacids, compounds which exert special regulatory effects on skeletal muscle protein catabolism and synthesis, and thus minimize net protein breakdown (17). While it is clear that BCAA (primarily leucine) can reduce net protein degradation in vitro, the effect of amino acid formulas supplemented with additional BCAA on skeletal muscle breakdown in catabolic patients remains controversial. For example, Freund and Cerra have administered solutions containing up to 45% BCAA and reported beneficial effects on plasma amino acid concentrations and nitrogen balance (19,20). In contrast, Daly and associates compared the effects of a standard amino acid solution containing 25% BCAA and a 45% BCAA enriched solution in a randomized, blinded study in postoperative patients. Nitrogen balance was comparable in the two groups of patients regardless of the composition of the amino acids infused (21).

The hypothesis that administration of BCAA diminishes the skeletal muscle catabolic response was tested by infusing varying concentrations of BCAA and studying skeletal muscle amino acid exchange and nitrogen metabolism following a standard catabolic stress.

1. Materials and Methods

Mongrel dogs were utilized in this experiment as previously described. The dogs weighed between 18 and 35 kg and underwent a preoperative muscle biopsy of the vastus lateralis to quantitate intracellular free amino acid levels. Five days later a standard laparotomy and retroperitoneal dissection were performed with the implantation of polyethylene catheters into the aorta and vena cava. During the 24-hour perioperative period, the dogs were infused via an external jugular catheter with either saline or different isonitrogenous amino acid solutions, containing increasing quantities of essential and decreasing quantities of non-essential amino acids. The BCAA varied in concentration between 12 and 42.4% of the total amino acids infused. Hindquarter amino acid flux was

determined at 6 and 24 hours postoperation, as previously described. A second muscle biopsy was obtained 24 hours postoperation. Urine was collected via an indwelling bladder catheter for the 24-hour perioperative period and was analyzed for total nitrogen content.

2. Results

Nitrogen balance was improved with amino acid infusion but only minimal differences were noted between the various solutions utilized (Table V). At 6 hours postop, nitrogen efflux (calculated as the sum of individual amino acid fluxes multiplied by their respective number of nitrogens) was reduced in all treatment groups compared to saline controls. BCAA were released from skeletal muscle during saline infusion, but hindquarter BCAA uptake occurred at 6 hours in all treatment groups and was related to BCAA concentration. At 6 hours hindquarter BCAA uptake correlated with skeletal muscle nitrogen retention. This effect was small and unrelated to nitrogen balance. At 24 hours hindquarter flux was similar in all groups. Skeletal muscle intracellular free amino acids were maintained at preoperative levels in all animals infused with amino acids but fell in the saline treated group.

3. Discussion

This standard operative procedure increases skeletal muscle nitrogen efflux six to eight times more than that which occurs in the basal, postabsorptive animal. In this catabolic model whole body nitrogen loss was minimized, skeletal muscle amino acid efflux diminished, and intracellular free amino acid concentrations preserved with amino acid infusions. Despite alterations in concentrations of BCAA from 12 to 42%, no major effects on total body nitrogen economy were observed. These results are similar to those findings of Daly et al. in catabolic surgical patients (21). Present available balanced intravenous formulas appear to maximize the anti-catabolic effects of amino acid administration.

IV. The Effect of Adrenergic Blockade on Skeletal Muscle Proteolysis

A. Introduction

With the stress of injury, or injury complicated by infection, a variety of neurohormonal responses occur. These changes characterized by a rise in circulating levels of glucagon, glucocorticoids, and catecholamines, are associated in time by a series of well recognized responses affecting the metabolism of carbohydrate, protein, and fat. Skeletal muscle proteolysis and negative nitrogen balance reflect loss of an important component of body protein that limits optimal function and may impair recovery. One method of minimizing skeletal muscle breakdown following injury is to provide adequate nutrients which will optimize net skeletal muscle synthesis during stress, and thus minimize skeletal muscle breakdown. This approach has been tested by evaluating the effects of administered nutrients on protein catabolism. The nutrients studied to date include "ketone" bodies (22) (see Annual Report #2)* and specialized amino acid formulations (see Section I.a of this report). Although effects of nutrient administration could be observed, such approaches were not without their own limitations (these include the energy costs of nutrients, transport, and metabolism, and the potential side effects of the therapy). Moreover, the particular advantages gained by administering balanced amino acid formulas occurred in large part because of the effects of the "mass balance" of nitrogen. The benefit(s) of amino acid administration on skeletal muscle metabolism were achieved by infusing relatively large quantities of nitrogen (4 grams protein equivalent/kg · day), which was accompanied by the increased urinary excretion of nitrogen. Even with amino acid administration, glutamine efflux from skeletal muscle remained accelerated. These data support nitrogen turnover studies performed in traumatized and septic patients which demonstrated that protein degradation was accelerated; feeding increased synthesis rates to approach or match accelerated rates of protein catabolic rates (23). The catabolic process continued unabated following feeding, but net body protein loss was minimized by food (nitrogen) intake.

* Annual Report , August 1981 - September 1982

Because of the limitations associated with the food intake and the lack of specific regulatory effects on protein metabolism in the catabolic period using nutrients such as ketone bodies or specialized amino acid formulas, we have attempted to define the role of specific mediators and modulators on the protein catabolic response to injury. Once these mediators can be identified and their role defined, it should be possible to modulate the protein catabolic response and evaluate the benefits of such treatment on protein catabolism, skeletal muscle function, and the eventual outcome of patients receiving such therapy.

Hormones exert major regulatory effects on protein metabolism. Of the counter-regulatory hormones, glucagon exerts its effects primarily on the liver and is not thought to mediate skeletal muscle proteolysis (24). Cortisone is known to increase following stress and is associated with accelerated gluconeogenesis and proteolysis (25). However, the administration of glucocorticoids alone does not account for the alterations in protein metabolism which occur following injury; synergistic effects of all three counter-regulatory hormones appear necessary to produce most of the posttraumatic responses commonly observed (26).

Catecholamines are elevated following injury and have been associated with posttraumatic hypermetabolism (27). Unlike most tissues of the body, however, skeletal muscle does not possess detectable sympathetic innervation. Yet, it contains adrenergic receptors and is responsive to adrenergic agents (28). It is possible that circulating catecholamines (primarily epinephrine) secreted by the adrenal medulla are major effectors. Also, norepinephrine released from sympathetic nerve endings may "spill over" into the bloodstream and mediate skeletal muscle adrenergic responses. Additional effects could result from neurotransmitters released from the innervation of the nearby smooth muscle cells of the skeletal muscle vasculature which diffuse through the local interstitial fluid compartment to affect skeletal muscle. Yet, another explanation of possible action is that catecholamines may activate prostaglandins in regional skeletal muscle beds (29). These compounds then serve as a (the) signal which initiates proteolysis (30).

Regardless of the source of the catecholamines and mechanism of action, adrenergic stimulation appears to be primarily responsible for some of the major alterations observed in skeletal muscle following injury. For example, dogs were infused with glucose to maintain fixed hyperglycemia (using the glucose clamp technique)

and then received isoproterenol, a β-agonist. Although insulin concentrations increased two-fold, total body glucose disposal was reduced to one-half mean control value. Skeletal muscle glucose disposal determined by simultaneous hindquarter flux was reduced 60% (31). In seriously injured patients the maximal glucose disposal rate during euglycemia and hyperinsulinemia was 9.17± 0.87 mg/kg · minute, significantly less than the 14.3±0.78 observed in the age-matched normal controls (32). Using forearm flux techniques a major portion of this tissue insulin resistance was localized to skeletal muscle (33). These total body and forearm changes have been simulated in normals with epinephrine infusion (34).

B. Epidural Anesthesia

While a number of techniques are available to block sympathetic nervous system outflow, one particularly applicable approach is high epidural anesthesia. This technique requires placement of a small catheter in the epidural space which is used to administer regional anesthesia. Utilizing this technique in patients undergoing abdominal hysterectomy, the elaboration of cortisol and catecholamines was significantly attenuated and many of the commonly observed features of the posttraumatic response blocked (35). In this study we administered high epidural anesthesia to our standard posttraumatic model to evaluate the effects of sympathetic blockade on posttraumatic responses.

Materials and Methods

Eleven mongrel dogs were studied. Approximately two weeks prior to study, six of the animals underwent anesthesia and operative placement of an epidural catheter. Using short acting general anesthetic (pentothal 0.3 mg/kg) and strict aseptic technique, a 2-cm vertical incision was made over the L7-S1 vertebrae and deepened until the ligamentum flavum was reached. A 17-gauge Tuohy needle was inserted into the forearm between L7-S1, and the bevel at the end of the needle positioned to allow the epidural catheter to be directed to the desired position. A 20-gauge teflon epidural catheter (Deseret, Utah) was passed through the needle and directed cranially until approximately 5 cm lay within the epidural space. This position was chosen because our early experiences showed it to be the most reliable for achieving anesthesia to the height desired while still maintaining the adequate caudal block. The catheter was tethered to the ligament by a silk suture to prevent displacement, and the suture was secured to the catheter with a drop of epoxy glue. A piece of sterile teflon tubing was used as an outer sleeve around the catheter to prevent kindking. The catheter was trimmed to the appropriate length and attached to

a blunt needle which, in turn, was connected to an intermittent injection port (Jelco). This was placed subcutaneously along the lateral aspect of the spinal column to allow repeated injection of anesthesia via percutaneous puncture. The skin wound was closed and the animal allowed to recover for at least 10 days. Three to five days prior to operation, biopsy of the vastus lateralis muscle was performed in all animals. On the day of study, all animals underwent general anesthesia, a standard abdominal exploration, retroperitoneal dissection, and implantation of aortic and caval catheters as previously described. The animals with the epidural catheters received epidural anesthesia before the induction of the general anesthesia.

Bupivacaine (Marcain, Sterling Drugs) 0.5% was the anesthetic agent used for epidural anesthesia because it possessed a relatively long duration of action. The dose required to produce anesthesia to the level of T4 was 0.3 ml/kg administered over 2-3 minutes; repeated doses of 0.15 ml/kg at 2-1/2 hour intervals were necessary for maintenance. Adequacy of anesthesia was judged by neurological assessment which confirmed loss of reflexes and sensation to the appropriate dermotomes. Barbiturate anesthesia was then administered and the standard operative procedure and catheter implantation performed in the manner previously described. In the postoperative period, assessment of the level of the epidural anesthesia was made at hourly intervals and additional anesthesia administered to maintain high epidural anesthesia, including the T4 through S1 segments, for 24 hours. Blood levels of cortisol and glucose were monitored to confirm the adequacy of the epidural blockade.

At 6 and 24 hours postoperation, all animals underwent hindquarter flux studies as previously described. At 24 hours a muscle biopsy was taken for amino acid analysis, as previously described, and the results compared with preoperative values. Urine was collected via a bladder catheter and urinary nitrogen quantitated.

Results

All animals receiving the epidural anesthesia were maintained without difficulty and tolerated the operative procedure and anesthesia well. In addition to the neurological signs of high epidural anesthesia (bilateral Horner's sign was frequently observed), blood glucose failed to rise following operation (6-hour glucose was 120 mg% in the control animals versus 90 mg% in the epidural group) and cortisol at 4 and 6 hours postoperative remained near normal

limits (4-6 μg/dL versus 16-25 μg/dL in controls). That both markers remained normal indicates an adequate postoperative sympathetic blockade (35).

At 6 hours postoperation, the blood amino acid nitrogen levels were similar in the epidural and saline control groups (Table VI). However, hindquarter nitrogen efflux was markedly reduced in the animals receiving epidural anesthesia, averaging less than 50% of the amino acid nitrogen released in controls. This was primarily due to the decrease in alanine flux; release of glutamine and the BCAA was similar between groups. Skeletal muscle intracellular concentrations of glutamine fell in both groups. In spite of the marked effect of epidural anesthesia on hindquarter flux, urinary nitrogen excretion was similar in both groups.

C. α- and β-Adrenergic Blockade

Combined adrenergic blockade was carried out in five animals. The blockade was initiated 30 minutes before operation and maintained throughout the 24-hour study period. Phentolamine was administered as the α-blocker (given as a 0.6 mg/kg loading dose followed by 0.45 mg/hr · kg constant infusion), and propranolol was used as the β-blocker (2.8 g/kg loading dose, 2.1 mg/hr · kg constant infusion). These doses have been shown to adequately block α- and β-responses in the dog (36).

Results

The blockade was tolerated well by the animals. At 6 hours whole blood amino acid nitrogen content was markedly reduced (Table VI). Hindquarter nitrogen efflux was also significantly less than the controls, and this was primarily reflected in a decrease in alanine release. Intracellular glutamine concentration diminished in three of the five animals studied and increased in the other two animals. In spite of these changes, however, urinary nitrogen loss was not significantly altered by the α- and β-blockade.

D. Discussion

This is the first report that net skeletal muscle proteolysis can be decreased following a major catabolic insult by altering communication between the sympathetic nervous system and skeletal muscle. Not all aspects of skeletal muscle catabolism were affected; glutamine efflux continued and intracellular glutamine concentration consistently fell. Simultaneously, the demand for alanine (presumably as a gluconeogenic precursor) was markedly reduced. That diminished net skeletal muscle breakdown occurred following adrenergic blockade is confirmed by reduced excretion of creatinine in the experimental animals. Creatinine is produced by muscle and reflects, in part, skeletal muscle breakdown rate (37). Creatinine excretion averaged 0.039±0.002 grams/day · kg in the control animals and fell to 0.030±0.002 and 0.031±0.001 in the two groups receiving adrenergic blockade ($p < 0.01$).

This occurred at a time when blood creatinine concentrations remained constant or fell during the perioperative period.

In spite of the ability to affect skeletal muscle proteolysis, urinary excretion of nitrogen remained elevated. This may be due to multiple mechanisms which affect hepatic gluconeogenesis and ureagenesis which were not affected by skeletal muscle adrenergic blockade. One such mediator is glucagon which has potent gluconeogenic effects and may be the mediator of the accelerated urea production. The nitrogen precursors for the urinary nitrogen excreted must have originated from other sources (visceral organs) or were derived from plasma amino acid (and possibly plasma proteins). This may have been the reason for the marked fall in the plasma amino acids in the animals undergoing α- and β-blockade. While further studies are necessary to determine the source of the excreted nitrogen and mediators of the visceral changes observed, the importance of these findings should be emphasized: skeletal muscle proteolysis can be diminished in the posttraumatic state by physiologic manipulation of the adrenergic nervous system.

V. The Effect of Epinephrine Infusion on Muscle Proteolysis and Intracellular Amino Acid Concentrations

Several studies indicate that the counter-regulatory hormones, cortisol, glucagon, and the catecholamines, affect the body in a manner which is consistent with what is found in injured patients. It has been hypothesized that the alteration in the hormonal milieu is the cause of the metabolic response to injury. It is believed that elevated levels of these hormones have a synergic effect on the body which causes accelerated muscle protein degradation, an efflux of amino acids from the periphery to the viscera, and increased hepatic uptake of amino acids and gluconeogenesis. This concept is gaining more support from *in vivo* studies in which investigators have infused these hormones into healthy humans and animals. It is theorized that such infusion will create a pseudo-injury state in healthy individuals if these hormones are responsible, at least in part, for the metabolic alterations that occur following injury. Sacca and his associates (38), from studies in which glucagon, epinephrine, and cortisol were infused into normal dogs, concluded that"changes in glucose metabolism in circumstances in which several counter-regulatory hormones are elevated (e.g. 'stress hyperglycemia') are a consequence of synergic interactions among these hormones." Bessey et al (26) found that infusion of these three hormones into healthy volunteers resulted in negative nitrogen balance. Comparison of these results with control studies in which normal saline was infused into subjects receiving the same nitrogen intake as the volunteers above received, indicates that the results were not due to

bedrest alone or the experimental conditions. Bessey and associates also found that whole-body protein catabolism was elevated relative to synthesis in volunteers receiving the hormonal infusion. Hence, present evidence supports the hypothesis that the synergism of the counter-regulatory hormones is a major mediator of the metabolic response to injury. This concept is supported by the findings of studies utilizing adrenergic blockade (Section IV) which demonstrate that skeletal muscle breakdown is diminished following interruption of communication between the sympathetic nervous system and skeletal muscle. This experiment evaluates the effect of infusion of epinephrine with or without other counter-regulatory hormones (glucagon and cortisol) on skeletal muscle amino acid metabolism.

Materials and Methods

Experimental Protocol

Male and non-gravid female dogs weighing between 20 and 35 kilograms were used. Each dog had been operated upon as previously described (Section III)for chronic implantation of aortic and vena caval catheters capped with subcutaneous injection ports. The dogs were fed Agway Respond Dry Dog Chow and received water ad libitum. All dogs used in the experiments had completely recovered from their operations, were healthy, and had been trained to rest quietly in the Pavlov stand.

Each dog was studied on two days separated by three to four days. On the night before each study day, food was withdrawn from the dogs at 5:00 p.m. so that they could be studied in the postabsorptive state. On the "Basal Study" day the dog was walked and then placed in a Pavlov dog stand, and temperature taken. Following this, the injection ports of the catheters were entered by subcutaneous puncture with a 21-gauge needle. The catheters were cleared with saline, and a venous blood sample was taken for measurement of plasma glutamine concentration and hematocrit determination. Finally, the dogs were anesthetized with sodium pentobarbital, and a needle biopsy of the vastus lateralis was taken for measurement of basal muscle intracellular glutamine concentration.

On the "Infusion Day" the dogs were treated as they were on the Basal Study day, up to and including the point where a venous blood sample was taken. After this, one of five infusions was begun and continued for 6 hours (see below). Venous blood samples were taken at two, four, and six hours from the start of the infusion for measurement of amino acids and glucose concentrations.

At six hours after commencement of the infusion, the dogs were anesthetized, and a needle biopsy of the vastus lateralis of the leg which had not been biopsied on the Basal Study day was taken. This was done for measurement of post-infusion muscle intracellular glutamine concentration.

Infusions

1. Isotonic saline (4 ml/kg dog weight . hour).

2. Isotonic saline with epinephrine (50 ng/kg dog weight . minute).

3. Isotonic saline with glucagon (3 ng/kg dog weight . minute).

4. Isotonic saline with epinephrine + glucagon (same doses as above).

5. Isotonic saline with epinephrine + glucagon (same doses as above) + cortisol (200 μg/kg dog weight . hour).

Results

With infusion, plasma glutamine concentration did not change with saline, epinephrine or glucagon alone (Table VII). Plasma glutamine concentration fell significantly with epinephrine plus glucagon or "triple hormones" (epinephrine + glucagon + cortisol). Muscle intracellular glutamine concentration tended to fall during the 6-hour study but did not change significantly with any treatment.

Because the major alterations appeared to be associated with the triple hormonal infusion, the alterations which occurred were evaluated in more detail (Table VIII). Hormonal infusion was associated with a fall in concentration of whole blood and intracellular amino acid nitrogen. During this same time period, plasma glucose rose from 111±5 mg/dl to 180±12 ($p < 0.001$). However, no alteration in efflux of hindquarter amino acids was noted with hormonal infusion; all flux values were indistinguishable from zero, suggesting that the hindquarter remained in amino acid balance during these experiments.

Discussion

These results demonstrate that some but not all of the metabolic effects observed in the postoperative model can be mimicked by the infusion of epinephrine in combination with other counter-regulatory hormones. The effects of hypoaminoacidemia must be due to accelerated

amino acid clearance by visceral tissues mediated via these hormones. Glucagon has been described as mediating amino transport into the liver; this effect appears augmented by epinephrine and cortisol.

In spite of these hormonal effects, posttraumatic hindquarter nitrogen efflux observed at 6 hours could not reproduce these changes in hormonal environment. While some skeletal muscle effects may have occurred (note decrease in skeletal muscle intracellular nitrogen), these changes could be explained by augmented skeletal muscle bloodflow which could lower intracellular concentrations via changes in concentration gradients and "washout effects."

This study does not support the hypothesis that epinephrine serves as the mediator of skeletal muscle proteolysis (in vitro studies suggest β-stimulation of skeletal muscle augments synthesis, not breakdown, of protein) (39), and coupled with the findings presented in Section 2, points to norepinephrine mediation as the stimulus for skeletal muscle catabolism.

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TABLE I

Plasma Amino Acid Concentration
(Mean±SEM)

Solution Infused	Preoperative			24 Hours Postoperative		
	Total Nitrogen (mmol/L)	GLN Conc (μmol/L)	Sum B.C.A.A. Conc (μmol/L)	Total Nitrogen (mmol/L)	GLN Conc (μmol/L)	Sum B.C.A.A. Conc (μmol/L)
Saline	4.51±0.37	845±99	326±21	4.56±0.29	742±60	501±9*
Amino Acids (0.625 gm N/24 hr·kg)	4.84±0.58	829±87	339±31	5.57±0.61	631±75	767±99**
Amino Acids + Glutamine (0.625 gm N/24 hr·kg)	3.86±0.29	643±36	297±31	5.92±0.49*	1042±88**	592±82*

* $p<0.01$, when compared to preoperative value by paired t-test.

** $p<0.001$, when compared to preoperative value by paired t-test.

TABLE II

Muscle Amino Acid Concentrations (Mean±SEM)
(Expressed as mmol/liter Intracellular Water)

	Preoperative					24-Hour Postoperative				
Solution Infused	Total Nitrogen (mmol/L)	GLN (mmol/L)	Sum B.C.A.A. (mmol/L)	Sum Essential A.A. (mmol/L)	Sum Non-Ess A.A. (mmol/L)	Total Nitrogen (mmol/L)	GLN (mmol/L)	Sum B.C.A.A. (mmol/L)	Sum Essential A.A. (mmol/L)	Sum Non-Ess. A.A. (mmol/L)
Saline	69.8± 8.5	21.48± 3.21	0.437± 0.017	1.81± 0.23	40.92± 4.38	52.8±** 8.4	15.86±* 3.80	0.591± 0.0165	1.90± 0.32	31.50± 4.70
Amino Acids (0.624 gm N/24 hr·kg)	65.2± 10.3	18.69± 3.74	0.471± 0.074	2.24± 0.29	39.00± 6.10	62.5± 9.6	18.20± 3.75	0.795± 0.144	2.77± 0.36	36.70± 5.40
Amino Acids + Glutamine (0.622 gm N/24 hr·kg)	63.5± 7.0	19.85± 3.17	0.442± 0.022	1.99± 0.29	37.50± 3.82	68.3± 4.4	21.65± 2.08	0.773±* 0.125	2.72± 0.22	39.74± 2.34

* $p<0.05$, when compared to preoperative values by paired t-test.

** $p<0.01$, when compared to preoperative values by paired t-test.

TABLE III

Hindquarter Nitrogen Flux
(Mean±SEM; μmol/min · kg)

	6-Hour Flux			24-Hour Flux		
	Total Amino Acid Nitrogen	GLN	B.C.A.A.	Total Amino Acid Nitrogen	GLN	B.C.A.A.
Saline	-19.05±4.06*	-2.69±1.07	-1.41±0.26**	-3.50±12.10	-1.71±0.70	+0.49±1.51
Amino Acids (0.624 gm N/24 hr·kg)	-7.70±5.90	-1.93±0.59	+1.62±0.86	-8.42±2.9	-1.24±0.44	+2.08±1.53
Amino Acids + Glutamine (0.624 gm N/24 hr·kg)	-6.52±1.81	-1.19±0.46	+0.28±0.15	-3.03±3.75	-0.16±0.82	+0.09±0.22

*$p<0.05$; saline different from all animals receiving 0.624 gm N/24 hr · kg.

**$p<0.01$, saline <amino acids + glutamine <amino acids.

- = release

+ = uptake

TABLE IV

Nitrogen Balance
(Mean±SEM)

Solution	Projected Nitrogen Intake (gm N/24 hr · kg)	N	Measured Intake	Excretion	Balance
			----------Nitrogen gm/24 hours · kg----------		
Saline	0	5	0	0.492±0.022*	-0.492±0.002**
Amino Acids***	0.312	2	0.304±0.002	0.637±0.056	-0.332±0.058
Glutamine	0.312	3	0.323±0.003	0.709±0.085	-0.386±0.086
Amino Acids***	0.624	6	0.632±0.001	0.684±0.031	-0.052±0.031
Amino Acids + Glutamine†	0.624	6	0.635±0.004	0.775±0.019	-0.140±0.022

* $p<0.05$; saline <0.624 gm N/24 hr · kg amino acids <0.624 amino acids + glutamine.

** $p<0.05$; saline <0.624 gm N/24 hr · kg amino acids + glutamine <0.624 amino acids

*** FreAmine III.®

† One-half of nitrogen provided by FreAmine and one-half by glutamine.

TABLE V

Effects of Varying Concentrations of Branched Chain Amino Acids on Nitrogen Metabolism
(Expressed as Mean±SEM)

BCAA Composition	N	Solution Infused	PRE-OP μmol/min · kg Nitrogen Intake	PRE-OP μmol/min · kg Nitrogen Balance	PRE-OP μmol/l Sum Plasma BCAA Conc.
0	5	Saline	0*	-0.492 ±.02*	335.0±21.9
12%	8	FreAmine III® + Nonessential AA	0.627 ±.006	-0.151 ±.02	283.9±25.9
21%	6	FreAmine III®	0.632 ±.001	-0.053 ±.03	339.2±30.8
42%	7†	FreAmine III® + BCAA + Nonessential AA	0.627 ±.003	-0.200 ±.05	262.8±11.0

BCAA Composition	SIX HOURS POST-OP μmol/l Sum Whole Blood BCAA Conc	SIX HOURS POST-OP μmol/min · kg Sum Hindquarter BCAA Flux	SIX HOURS POST-OP μmol/min · kg Hindquarter Nitrogen Flux	24 HOURS POST-OP μmol/l Sum Plasma BCAA Conc	24 HOURS POST-OP μmol/min · kg Sum Hindquarter BCAA Flux	24 HOURS POST-OP μmol/min · kg Nitrogen Flux
0	294.0 ±17.6	-1.14±0.26	-19.05 ±4.06*	500.7 ±8.6	+0.49±1.51	-3.50 ±12.1
12%	455.0 ±57	+0.47±0.29	-6.86 ±1.35	609.0 ±61	+0.17±0.20†	-4.43 ±2.43
21%	637.0 ±67	+1.62±0.86	-7.67 ±5.90	767.0 ±99	+2.09±1.54	-8.42 ±2.90
42%	961.0 ±76	+1.96±0.25	-1.79 ±2.08	1390.8 ±47.8	+1.91±0.99	-8.10 ±8.6

* $p<.05$, when saline compared to all others (no difference between amino acid treatment groups except nitrogen balance for 21% BCAA which is significantly less negative than 12% or 42%).

† N = 4, for flux values, and blood concentrations.

\+ = uptake.

\- = release.

TABLE VI

Effect of Adrenergic Blockade on Nitrogen Metabolism Following Operation

Treatment	N	24-Hour Nitrogen Excretion gm N/day.kg	Whole Blood Amino Acid Nitrogen mmol/L	6-Hour Flux: Hindquarter N Flux µM/min · kg	6-Hour Flux: Glutamine Flux µM/min · kg	6-Hour Flux: Alanine Flux µM/min · kg	6-Hour Flux: BCAA Flux µM/min · kg
Controls	5	0.492±0.02	3.82±0.08	-19.05±4.06*	-2.68±1.06	-2.19±0.52*	-1.13±0.26
Epidural Anesthesia	6	0.553±0.03	3.95±0.06	-8.89±0.86	-1.08±0.28	-1.25±.41	-0.65±0.52
α+β Blockade	5	0.489±0.06	3.07±0.13*	-5.54±1.55	-0.46±0.66	-0.77±0.15	-0.37±0.11

*$p<0.05$, different from other two groups by analysis of variance.

**Whole blood nitrogen content in the animals before operation averaged 4.720±0.21 mmol/L.

- = release.

+ = uptake.

TABLE VII

The Effect of Hormonal Infusion on Plasma and Muscle Intracellular Glutamine Concentrations in the Dog (Mean±SEM)

		PLASMA (μmol/L)		MUSCLE (mmol/L)	
	N	Before	After	Before	After
Saline	7	879±98	893±54	18.07±1.71	15.33±1.4
Epinephrine	2	727±211	691±111	24.28±7.16	20.91±4.56
Glucagon	4	843±113	753±121	23.62±3.45	25.40±1.38
Epi + Glucagon	6	673±55	514±57*	20.56±3.10	18.65±1.85
Epi + Glucagon + Cortisol	7	771±57	467±49**	18.81±1.75	14.05±2.90***

*$p<0.05$, different from before by paired t-test.

**$p<0.001$, different from before by paired t-test.

***$p<0.054$, different from before by paired t-test.

TABLE VIII

The Effects of Infusion of Saline or Counterregulatory Hormones Over 6 Hours into "Normal" Dogs

		WHOLE BLOOD				SKELETAL MUSCLE INTRACELLULAR AMINO ACIDS				HINDQUARTER FLUX (μm/min)			
		Amino Acid Nitrogen (mM)		Glutamine (μM)		Amino Acid Nitrogen (mM)		Glutamine (μM)		Amino Acid Nitrogen Flux		Glutamine Flux	
	N	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Saline	7	5.60± 0.28	5.90± 0.02	859±68	896± 38	69.86± 3.73	58.50 +5.40	18.07± 1.71	15.33 ±1.40	-3.99± 9.70	-9.20 ±5.2	-0.36± 4.62	-2.77± 1.84
		NS		NS		NS		NS		NS		NS	
Epi + Gluc + Cortisol	7	5.31± 0.17	3.73± 0.20	743±53	493± 26	68.88± 5.93	46.88 ±8.96	18.81± 1.75	14.04 ±2.90	+0.95± 5.20	-11.05 ±12.40	+1.56± 1.51	+0.33 ±1.28
		p<0.001		p<0.05		p<0.05		NS+		NS		NS	

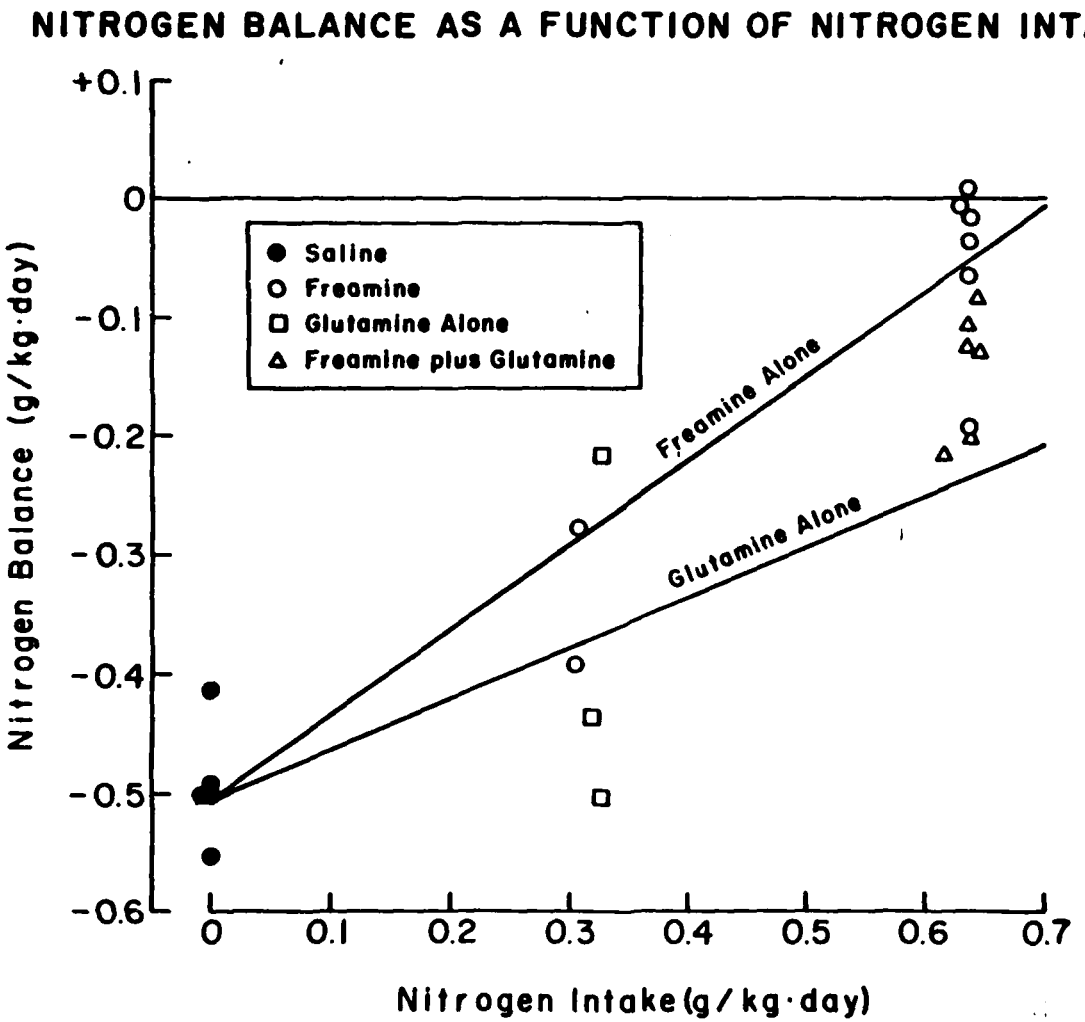
+ p=0.54

FIGURE I

The Relationship Between Nitrogen Intake and Nitrogen Balance is Expressed as:

$N_{Bal} = -0.506 + 0.712\ FreAmine + 0.418\ glutamine$

All Units in gm/kg · day, $p<0.05$, $R^2 = 0.85$.



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